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PIEBALDISM IN DIAMOND–BLACKFAN ANAEMIA: A NEW PHENOTYPE?

Piebaldism is a rare autosomal dominant disorder characterized by a defect in pigmentation revealed as bilateral but non-symmetrical white patches of hair and skin mostly affecting the face, the chest, the abdomen and the extremities, but never the back. The similar pigmentation phenotype of dominant White spotting mice (Geissler *et al*, 1988) and human piebaldism enabled the characterization of the gene responsible for the piebald trait in both species. *c-kit* gene was found mutated and associated with piebaldism in both human and mice (Reith *et al*, 1990). Although mutations in stem cell factor (SCF) gene in Steel (*Sl*) mice (Copeland *et al*, 1990) reproduce the dominant White spotting mice phenotype, to date, no mutation in SCF gene has been identified in human piebaldism. While piebaldism in human is the only clinical manifestation reported in association with mutations in the *c-kit* gene, in the mouse, haematopoiesis and gametogenesis are also affected to varying levels depending both on the nature of mutation in *c-kit* gene and on the homozygosity or heterozygosity of the mutation (Reith *et al*, 1990). Dominant White spotting (W) and Steel (*Sl*) mice (Geissler *et al*, 1988) exhibit mild to severe macrocytic anaemia, reticulocytopenia, and normal platelet and granulocyte counts, which are phenotypic anomalies similar to those observed in Diamond–Blackfan anaemia (DBA). Extensive studies failed to reveal mutations in either the *c-kit* gene or its ligand SCF in association with DBA phenotype (Spritz & Freedman, 1993). However, recent studies have identified mutations in the ribosomal protein S19 (*RPS19*) gene in 25% of patients affected by DBA (Willig *et al*, 1999), and a yet to be characterized gene on chromosome 8p has been also linked to DBA phenotype (Gazda *et al*, 2001). With the identification in the French DBA registry of a patient carrying piebaldism, we wondered whether a mutation in the *c-kit* gene could explain both the piebald trait and erythroblastopenia or if piebaldism was part of the DBA phenotype. To distinguish between these two alternatives, we sequenced all 21 exons of *c-kit* gene and all nine exons of SCF gene. Our failure to document any mutation in either the *c-kit* gene or in the SCF gene implies that other gene defects are responsible for both DBA and piebaldism phenotypes in our patient, and piebaldism was not related to a mutation in *c-kit* or SCF genes. Although we could not eliminate a neomutation, another argument in favour of this conclusion is the fact that our patient is the only one in his family to carry a piebald trait despite the known autosomal dominant inheritance of piebaldism. This finding, which to our knowledge is the first such case described in DBA, extends piebaldism as a potential feature associated with DBA.

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Keywords: Diamond–Blackfan anaemia, piebaldism, *c-kit*, SCF.

SUCCESSFUL UMBILICAL CORD BLOOD TRANSPLANTATION IN A CHILD WITH DYSKERATOSIS CONGENITA AFTER A FLUDARABINE-BASED REDUCED-INTENSITY CONDITIONING REGIMEN

Dyskeratosis congenita (DC) is characterized by atrophy and reticular pigmentation of the skin, nail dystrophy and leukoplakia of mucosa membranes (Dokal, 2000). Other manifestations include hypotricosis, dysphagia, skeletal abnormalities, mental retardation and bone marrow failure (Dokal, 2000). Despite the fact that DC is considered mainly to be an X-linked recessive disorder, cases of both autosomal recessive and dominant transmission have been reported (Dokal, 2000). Allogeneic haematopoietic stem cell transplantation (HSCT) remains the only curative approach for severe bone marrow failure experienced by patients with DC. However, results of allograft in these patients have been relatively poor, due to occurrence of both early and late complications, reflecting the increased sensitivity of endothelial cells to radiotherapy and alkylating agents (Berthou *et al*, 1991; Langston *et al*, 1996). As interstitial and obstructive lung disease, as well as liver toxicity, have been observed (Rocha *et al*, 1998), conditioning regimens avoiding radiotherapy and busulphan have been suggested. We report a patient with DC developing aplastic anaemia who was given cord blood transplantation (CBT) from a human leucocyte antigen (HLA)-identical sibling, using a fludarabine-based non-myeloablative conditioning regimen. A boy was diagnosed with DC at the age of 2 years. He was found

Table I. Summary of haematopoietic stem cell transplantation in DC patients.

Patient number	Age (years)/ sex	Sibling	Source of Stem cells	HLA	Conditioning regimen	Outcome
1	33/M	YES	BM	6/6	CY 200 mg/kg	Died of liver failure 51 d after BMT
2	4/F	YES	BM	6/6	CY 240 mg/kg and Bu 8 mg/kg	Died of gastrointestinal bleeding 3 months after BMT
3	6/M	YES	BM	6/6	CY 120 mg/kg and TBI 7 Gy	Died of liver failure 8 years after BMT (diagnosis of VOD confirmed by autopsy)
4	11/M	YES	BM	6/6	CY 150 mg/kg and TAI 6 Gy	Died of cerebral haemorrage 2 years after BMT
5	29/M	NO	BM	6/6	CY 200 mg/kg	Rejection. After 2^ BMT died of respiratory failure
6	2/M	YES	BM	6/6	CY 200 mg/kg	Alive (5 years after BMT)
7	8/M	YES	BM	6/6	?	Alive (18 months after BMT)
8	3/F	YES	BM	6/6	CY 200 mg/kg	Died of pulmonary fibrosis 20 years after BMT
9	11/F	YES	BM	6/6	CY 200 mg/kg	Died of pulmonary fibrosis 8 years after BMT
10	8/M	YES	BM	6/6	CY 200 mg/kg	Died of aspergillosis 7 d after BMT
11	26/M	YES	BM	6/6	CY 200 mg/kg	Died of pulmonary fibrosis 70 d after BMT
12	33/M	YES	BM	6/6	CY 140 mg/kg and ATG	Alive (463 d after BMT)
13	22/M	YES	BM	6/6	CY 140 mg/kg and ATG	Died of acute GVHD 44 d after BMT
14	23/M	NO	BM	6/6	CY 120 mg/kg and TBI 12 Gy	Died of candidiasis 13 d after BMT
15	20/M	NO	BM	6/6	CY 120 mg/kg and TBI 12 Gy	Died of candidiasis 14 d after BMT
16	2/M	YES	BM	DP mismatch	CY 200 mg/kg and TAI 3 Gy	Died of pulmonary fibrosis 7 years after BMT
17	10/M	YES	BM	6/6	CY 150 mg/kg and TAI 6 Gy	Died of liver failure 8 years after BMT
18	5/M	YES	BM	6/6	CY 200 mg/kg	Alive (7·5 years after BMT). Karnofsky 80%; chronic respiratory failure; malabsortion
19	10/M	YES	BM	Mother DP mismatch	CY200 mg/kg; Bu 6 mg/kg and ATG	Died of invasive aspergillosis 1·5 years after BMT
20	18/M	YES	BM	6/6	CY 80 mg/kg and Bu 0,8 mg/kg	Alive (6 years after BMT)
21	21/F	YES	BM	6/6	CY 80 mg/kg and Bu 0,8 mg/kg	Alive (5 years after BMT)
22	9/M	YES	BM	6/6	CY 200 mg/kg and ATG	Alive (4 years after BMT)
23	8/M	YES	CB	6/6	CY 40 mg/kg; Fludarabine 120 mg/m^2 and ATG	Alive (3 years after CBT)

GVHD, graft-versus-host disease; CY, cyclophosphamide; Bu, busulphan; TBI, total body irradiation; TAI, thoracoabdominal irradiation; ?, unknown; ATG, antithymocyte globulin; BMT, bone marrow transplantation; CBT, cord blood transplantation. Patient 23 is reported in this paper. Details of the original reports of the other cases included in this table can be obtained from the authors.

to have skin reticular pigmentation, nail dystrophy, microcephaly, hyperhydrosis, hypotricosis and short stature. At that time, full blood cell count showed haemoglobin 3·1 g/dl, white blood cell count $3{\cdot}0 \times 10^9$/l (absolute neutrophil count, ANC, $1{\cdot}0 \times 10^9$/l) and a platelet count of 27×10^9/l. Bone marrow aspirate showed tri-linear hypoplasia and *in vitro* culture of committed progenitors documented reduced colony growth. Chromosomal analysis of both peripheral blood and marrow cells was normal and the diepoxybutane (DEB) test was negative. As anaemia and thrombocytopenia persisted, he was transplanted 5 years after diagnosis using the placental blood from his HLA-identical sister. The conditioning regimen consisted of 4 d (d −6 to d −3) fludarabine (30 mg/m^2/d), cyclophosphamide (10 mg/kg/d) and rabbit antithymocyte globulin (ATG 3·75 mg/kg/d). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin A. The number of nucleated cells infused was 3×10^7/kg. The post-transplant course was uneventful; ANC > $0{\cdot}5 \times 10^9$/l was reached on d +18 and a self-sustained platelet count > 20×10^9/l on d +40. No symptoms or signs of either acute or chronic GVHD were observed. Complete donor chimaerism was documented on d +20, and 3, 6, 12 and 24 months after SCT. Thirty-six months after CBT, he is well, with normal blood count, normal immune function and a Lansky score of 100%.

Effectiveness of allogeneic HSCT in patients with DC has been hampered by propensity to develop early and late complications. To the best of our knowledge, only seven out of 22 patients given an allograft have survived, fatal late events that occur years after transplantation have been reported (Berthou *et al*, 1991; Langston *et al*, 1996; Rocha *et al*, 1998; Ghavamzadeh *et al*, 1999) (Table I). Endothelial damage/activation syndrome (liver venous occlusive disease, thrombotic thrombocytopenic purpura) and pulmonary fibrosis have been reported to be common and often lethal events due to increased sensitivity of endothelial cells to both radiation and alkylating agents. Thus, it has been suggested that the preparative regimen should include neither radiotherapy nor busulphan (Rocha *et al*, 1998; Dokal, 2000). Fludarabine has been used successfully for allogeneic HSCT in patients not eligible for conventional conditioning, as regimens including this drug are well tolerated and have limited extra-medullary toxicity. We reasoned that a preparative regimen comprising fludarabine, low-dose cyclophosphamide and ATG should be sufficient to enable engraftment of donor stem cells without undue toxicity. As patients given a cord blood transplant have a low risk of developing GVHD (Rocha *et al,* 2000), we also speculated that use of this source of stem cells should spare our patient from the effects related to occurrence of immune-mediated complications, which represent a contributing factor for post-transplant endothelial damage (Rocha *et al*, 1998). Our case suggests that regimens including fludarabine may represent a safe and efficacious approach to cure aplastic anaemia in DC patients, in whom donor healthy stem cells should have a growth advantage.

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Keywords: dyskeratosis congenita, cord blood transplantation, fludarabine, reduced-intensity conditioning regime.

HYPERBARIC OXYGEN FOR CYCLOPHOSPHAMIDE-INDUCED CYSTITIS

Haemorrhagic cystitis is a recognized complication of cyclophosphamide chemotherapy that may occasionally be severe or even fatal. Treatment is notoriously difficult and often unsatisfactory, prompting a search for new therapeutic alternatives. Hyperbaric oxygen (HBO_2) has now been used successfully in the treatment of radiation-induced haemorrhagic cystitis. It may also be useful in chemotherapy-induced haemorrhagic cystitis as the histological changes are similar in radiation- or chemotherapy-induced damage, with diffuse mucosal oedema, telangectasia and submucosal haemorrhage.

We describe here an 82-year-old gentleman who developed refractory severe haemorrhagic cystitis secondary to cyclophosphamide chemotherapy prescribed for myeloma. Initial treatment with melphalan failed to control his paraprotein and therapy was switched to weekly oral cyclophosphamide at a dose of 600 mg. Four months later, he developed frank haematuria, clot retention and bladder spasms. Cyclophosphamide was discontinued. Haematological results showed marked anaemia and thrombocytopenia [Hb 5·5 g/dl, white blood cell count (WBC) $2{\cdot}6 \times 10^9$/l, neutrophils $2{\cdot}1 \times 10^9$/l, platelets 49×10^9/l]. Coagulation profile was normal. Urethral catheterization and bladder irrigation failed to settle the haematuria. Flexible cystoscopy showed diffuse bladder wall oedema and haemorrhage consistent with cyclophosphamide-induced haemorrhagic cystitis. Despite the addition of oral tranexamic acid and bladder irrigation by alum 1% solution, gross haematuria and bladder spasms continued, and he remained heavily transfusion dependent, requiring 12 units of red blood cells over a 2 week period to maintain a Hb of 9·0–10·0 g/dl. Platelet counts were stable around 50×10^9/l without support.

At this point, referral was made to the hyperbaric medicine service. Thirty-eight treatments were given in a multiplace recompression chamber. One hundred per cent oxygen was administered 5 d per week for 90 min at a pressure of 200 kPa (31 treatments) or 240 kPa (seven treatments), and during a 30 min depressurization. Bilateral myringotomies were necessary as he found it difficult to cope with equalizing pressure across his tympanic membranes. His drums had healed by the end of the course. After 3 weeks of HBO_2 therapy, his transfusion requirements had fallen to 2 units of red blood cells every 5 d. The haematuria cleared completely 5 weeks after the start of therapy. The urinary catheter was removed prior to discharge with preservation of normal bladder function. This gentleman remains well with no recurrence of haematuria 12 months later.

To date, there have been six other reports of chemotherapy-related haemorrhagic cystitis that have been successfully treated with HBO_2 (Shameem *et al*, 1992; Yazawa *et al*, 1995; Hughes *et al*, 1998; Furness *et al*, 1999; Hattori *et al*, 2001). All six patients, aged 18 months to 41 years, received cyclophosphamide as part of a chemotherapy regimen or as conditioning prior to transplant. Two patients were also found to have adenovirus and adenovirus with BK virus in their urine. Conservative treatment was unsuccessful and referral to a hyperbaric unit was made at a mean of 47 d after the onset of symptoms. Each patient received 100% oxygen for 60–90 min at 200 kPa 5–7 d per week. Complete clearance of their urines occurred after 14–40 treatments. Follow-up data exists for four of the patients, with urine remaining clear at 14–28 months.

HBO_2 is a relatively safe, non-invasive but moderately expensive therapy. It is thought that the increased oxygen partial pressure diffusion gradient between the circulation and surrounding tissues enhances neutrophil function and macrophage migration into the damaged soft tissue promoting collagen formation, fibroblast growth and neoangiogenesis. The commonest complication of treatment is middle ear and/or sinus barotrauma, with 10% of (non-diving) patients in our unit requiring unilateral or bilateral myringotomy to facilitate pressurizations. We have not observed oxygen convulsions at pressure of 240 kPa or less in 22 years of operation.

Where available, we would recommend that HBO_2 be considered in the management of intractable chemotherapy-induced haemorrhagic cystitis.

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Keywords: haemorrhagic cystitis, hyperbaric oxygen, cyclophosphamide.

SEVERE INCREASE IN CREATININE WITH HYPOCALCAEMIA IN THALIDOMIDE-TREATED MYELOMA PATIENTS RECEIVING ZOLEDRONIC ACID INFUSIONS

We have recently introduced the new, highly potent, bisphosphonate, zoledronic acid (Zometa; Novartis, Frimley, UK) in place of pamidronate as a 'maintenance' treatment for our myeloma patients with osteolytic lesions. Its use has been investigated in the treatment of tumour-induced hypercalcaemia and in the prevention of skeletal complications associated with bone lesions in patients with breast cancer, multiple myeloma, prostate cancer and solid tumours (Major *et al*, 2001; Rosen *et al*, 2001). It is excreted via the renal route, but product information based on clinical research indicates that no dose adjustment is necessary in patients with mild to moderate renal impairment: serum creatinine < 400 μmol/l (Novartis). Creatinine clearance (CC) was defined as normal if greater than 1·33 ml/s; mild renal impairment if CC 0·83–1·33 ml/s; and moderate to severe if CC was between 0·16 and 0·83 ml/s. There was no dosing recommendation for patients with CC below 0·5 ml/s. We, therefore, did not include patients with CC of less than 0·5 ml/s. We also excluded patients with a creatinine > 250 μmol/l and any patients with calcium levels below the normal range.

We commenced Zometa for a trial period in a group of 16 patients with advanced myeloma and osteolytic lesions at a standard dose of 4 mg, given in 100 ml 0·9% saline over 15 min. Zometa may be infused over a 5–15 min period, depending on the renal function. We chose to use the longer time period for all our patients. These patients had all previously been maintained on a monthly dose of 60–90 mg of pamidronate for at least 1 year with no clinical or biochemical problems. The reasons for change included: greater convenience for patients and staff, with shorter infusion times and a more potent effect on bone disease, and possibly additional biological activity against myeloma with no increase in cost. The range of pretreatment serum creatinine levels in the 16 patients was 59–200 μmol/l, median 93 μmol/l. The calculated creatinine clearance of this group was 0·5–1·7 ml/s, median 1·125 ml/s. The patients received three doses of Zometa, their creatinine and calcium levels being monitored between each dose. Eleven of the patients were receiving thalidomide as their primary myeloma treatment, the remaining five were on either melphalan and prednisolone (three) or not on other myeloma treatment at that time (two).

We report two cases of dramatic rise in creatinine and associated hypocalcaemia following the switch to Zometa in patients who were previously stable on pamidronate.

CASE 1

A 74-year-old woman was diagnosed with myeloma in 1998. At that time, she had an IgG^{κ} paraprotein of 17 g/l, creatinine of 117 μmol/l and corrected calcium of 2·44 mmol/l. She initially showed a good response to melphalan with a plateau phase of 1 year. By November 2000, her paraprotein had risen to 19 g/l. Her creatinine at that time was 141 μmol/l and calcium 2·45 mmol/l. She commenced weekly cyclophosphamide and monthly pamidronate (60 mg). Eight months later, thalidomide (200 mg once daily) was substituted for cyclophosphamide. In 2 months, the patient had a dramatic reduction in paraprotein from 15·5 to 6·6 g/l. The last dose of pamidronate was given in September 2001, with creatinine 129 μmol/l, CC of 0·5 ml/s and calcium of 2·26 mmol/l. She received three doses of Zometa at monthly intervals following this. The thalidomide dose was reduced to 100 mg after the first dose of Zometa as the patient complained of tremor. However, the serum creatinine at that time was stable, but with a borderline calcium of 2·16 mmol/l. After two further doses of Zometa, and with a falling paraprotein of 5 g/l, repeat electrolyte analysis showed a creatinine of 331 μmol/l (CC 0·18 ml/s) and a calcium of 1·13 mmol/l. The patient felt shaky with paraesthesiae and generalized weakness. She had been taking diclofenac 50 mg t.d.s. continuously since diagnosis, and this was stopped, along with the thalidomide, in view of the deteriorating renal function. She was treated with intravenous fluids and calcium gluconate and subsequently with calcitriol. Her creatinine peaked at 380 mmol/l, but is slowly improving, and was 199 μmol/l at the time of writing, 3 months after the last dose of Zometa. The serum calcium level is still low at this stage, requiring continuing supplements (1·9 mmol/l, 12 weeks after the last infusion). An ultrasound scan was performed, results being consistent with the development of acute renal failure (ARF). Urine microscopy and culture excluded the presence of an urinary infection.

CASE 2

A 68-year-old man was diagnosed with myeloma in 1999. He had an IgG^{κ} paraprotein of 35 g/l, creatinine of 100 μmol/l and calcium of 2·28 mmol/l. His disease was unresponsive to melphalan (four courses) and, from November 1999, he received weekly cyclophosphamide and monthly pamidronate (90 mg). Following this, the paraprotein stabilized at 23 g/l and thalidomide (100 mg daily) was introduced. This produced a further reduction in paraprotein to 6·9 g/l over the following 12 months. Despite the disease response, the serum creatinine had, however, deteriorated over this time to 204 μmol/l (CC 0·58 ml/s) and the thalidomide dose was reduced to 50 mg. The creatinine remained stable over the next 6 months, with a calcium of 2·36 mmol/l, and Zometa was substituted for pamidronate. There was no initial change in renal function, but following the third dose, the creatinine level had risen to 482 μmol/l (CC 0·245 ml/s) with a calcium of 1·76 mmol/l. His thalidomide and long-term bendrofluazide were discontinued and intravenous fluids commenced. The creatinine peaked at 517 μmol/l but then fell over the

next 3 d to 358 μmol/l. The serum calcium again remained below the normal range requiring supplements for 6 weeks after the last dose of Zometa.

Again, ultrasound scan demonstrated no obstructive lesion, but changes consistent with acute and chronic renal impairment. In addition, the patient had negative urine cultures.

One further patient, also receiving thalidomide had a rise in creatinine from 150 μmol/l to a peak of 239 μmol/l, again after the third dose, but calcium levels in this patient remained within the normal range, and the creatinine quickly decreased to pre-Zometa levels on discontinuation.

The creatinine clearances for the patients also on thalidomide were as follows: median 1·0 ml/s; 1·33+ ml/s, three patients; 0·83–1·33 ml/s, four patients; 0·5–0·83 ml/s, four patients. The median CC for the five patients not on thalidomide was 1·12 ml/s; 1·33+ ml/s, one patient; 0·83–1·33 ml/s, three patients; 0·5–0·83, one patient.

CONCLUSION

Zometa is a new, highly potent bisphosphonate that has demonstrated to have between 40- and 850-fold greater potency than pamidronate in preclinical models of bone resorption (Green *et al*, 1994). The bisphosphonate has been thoroughly assessed in terms of efficacy and safety when compared with standard-dose pamidronate (90 mg) in patients with advanced myeloma and osteolytic bone lesions, and breast cancer patients (Berenson *et al,* 2001; Rosen *et al*, 2001) with licence for this indication imminent in the UK in addition to current widespread use as a 'maintenance' treatment in the USA and Germany, as well as its licensed use in the UK for tumour-related hypercalcaemia.

The above studies reported the incidence of renal impairment among patients treated with 4 mg of Zometa as similar to that among patients treated with pamidronate. Fifteen minute infusion times were used in the study by Rosen *et al* (2001). Grade 3–4 increases in creatinine were reported in 1% of both Zometa and pamidronate groups. Berenson *et al* (2001) also demonstrated a 1·5% incidence of hypocalcaemia in the Zometa group. These results are in marked contrast to our current result of 12·5%. The drug is becoming widely used in the USA and Germany for this indication. There are only a few reports of significant renal impairment with its use, but there are early indications of a possible link with concurrent use of thalidomide. Our group of 16 patients had all been stable on pamidronate infusions, and the two cases reported had received monthly infusions for 1 and 2 years respectively. All were given the Zometa dose over the maximum suggested time of 15 min. Both these cases had disease responsive to thalidomide at the time of the deterioration in renal function. In our second case, there had been a previous decline in renal function, but this had stabilized for several months prior to the change of bisphosphonate. Both cases were on thalidomide, which very occasionally may be the cause of renal impairment (drug information leaflet for American product; no recorded reports in Britain to our knowledge). In addition, one patient was on a non-steroidal drug and the other on a thiazide diuretic. The product guide for Zometa states that 'Zometa has been administered concomitantly with … diuretics, antibiotics and analgesics without clinically apparent interactions occurring.' These latter drugs (diuretic and non-steroidal) had been administered through the whole course of each patient's illness without prior adverse effect, with no alteration in dose and without apparent interaction with pamidronate and in the context of responsive disease. In both patients, an ultrasound scan was performed to rule out renal obstruction, and urinary tract infection was also excluded. Therefore, although the causes of the sudden, dramatic decline in renal function may be multifactorial in these cases, the temporal relationship to Zometa infusions and the possible interaction with thalidomide raise serious concerns about the use of these agents in combination. Thalidomide is being used increasingly in advanced myeloma and, as the use of Zometa becomes more widespread, clinicians should exercise caution and ensure careful biochemical monitoring, including creatinine clearance. Myeloma patients, who have vulnerable renal function, may require less frequent dosing intervals, although the recent studies mentioned used a monthly schedule. Until further data is available, we have returned to the use of pamidronate as our bisphosphonate of choice in this setting.

We recommend further study to determine whether patients with myeloma and impaired renal function require an alternative dose schedule of zoledronic acid.

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Keywords: Zometa, zoledronic acid, creatinine, hypocalcaemia, thalidomide.

TREATMENT OF REFRACTORY AUTOIMMUNE (ACQUIRED) HAEMOPHILIA WITH ANTI-CD20 (RITUXIMAB)

The recent letter from Ahrens *et al* (2001) concerning the use of rituximab in refractory autoimmune haemolytic anaemia is one of an increasing number of reports of the use of this monoclonal antibody in disorders of humoral autoimmunity. We report a case of autoimmune (acquired) haemophilia resistant to conventional immunomodulatory therapy that has responded to rituximab. Rituximab is a chimaeric mouse/human anti-CD20 monoclonal antibody developed and licensed for the treatment of CD20-positive lymphomas. Over the last few years, this has been used in a number of conditions which are primarily immune-mediated, including immune thrombocytopenia (Stasi *et al,* 2001), cold agglutinin disease (Sparling *et al*, 2001), autoimmune haemolytic anaemia (Ahrens *et al,* 2001) and IgM-mediated neuropathy (Levine & Pestronk, 1999). There have been studies presented as abstracts (Karwal *et al,* 2001; Weistner *et al,* 2001) but no published reports of the use of rituximab in managing the difficult and often expensive problem of factor VIII inhibitors.

Our patient initially presented in 1991 at the age of 18 years with a bleed into the right calf. He was found to have a prolonged activated partial thromboplastin time, factor VIII clotting level < 1% and factor VIII:C inhibitor level of 600 Bethesda units. Temporary immunosuppression was achieved with prednisolone and, later, cyclophosphamide. Subsequently, he became refractory to all forms of immunosuppression, including cyclophosphamide, prednisolone, azathioprine, cyclosporine and intravenous gammaglobulin. Frequent bleeds over the years were treated with porcine factor VIII, to which he became refractory, and prothrombin complex concentrates. Since 1996, recurrent soft tissue bleeds were treated with recombinant factor VIIa, tranexamic acid or prothrombin complex concentrates.

In May 2001, after giving informed consent, he received rituximab at 375 mg/m^2 once weekly for 4 weeks. He was on no other immunosuppression over that period, or subsequently. Over the next 4 months the inhibitor level fell from 268 Bethesda units to < 1 Bethesda unit. In April 2002 the inhibitor level remains < 1 Bethesda unit. He has had no significant bleeding episodes since administration of the rituximab.

CD20 is a B cell-lineage antigenic expressed from pre-B cells to mature and activated B cells. Its expression usually wanes as B cells differentiate towards plasma cells (Banchereau *et al*, 1992). Rituximab causes B-cell depletion in lymph nodes, peripheral blood and bone marrow. In autoimmune disease it may work to eliminate autoreactive clones. In our patient there was a marked decrease in circulating inhibitor levels and in symptomatic bleeding episodes following the course of rituximab.

In the year prior to rituximab therapy, this patient had been admitted on four occasions with severe bleeding episodes treated with recombinant factor VIIa. The estimated cost of these treatments was $A823 000. Since the rituximab treatment, which cost $A15,000, he has had no significant bleeds and the inhibitor has disappeared.

Factor VIII inhibitors in both acquired and genetic forms of haemophilia may be difficult and expensive to manage. There is a significant morbidity and mortality associated with these inhibitors. Further studies are needed to confirm whether rituximab has a definite role in the early treatment of cases unresponsive to steroids and other immunosuppresive chemotherapy. If so, there would be a significant reduction in the cost of management and improvement in the quality of life of patients with clinically significant FVIII inhibitors.

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Keywords: rituximab, refractory autoimmune (acquired) haemophilia, factor VIII inhibitor.

IS HIGH EXPRESSION OF THE CHEMOKINE RECEPTOR CXCR-4 OF PREDICTIVE VALUE FOR EARLY RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA?

We were very interested in the paper of Crazzolara *et al* (2001), which indicated that overexpression of the chemokine receptor CXCR-4 on malignant acute lymphoblastic leukaemia (ALL) cells is associated with extramedullary organ infiltration. Therefore, we were prompted to analyse the expression of CXCR-4 on blast cells at diagnosis of 10 children with an extramedullary relapse (testis or central nervous system, with or without medullary relapse) [median white blood cells (WBC) at diagnosis: 30×10^9/l; median disease-free survival (DFS): 34 months]. The results were compared with the expression of CXCR-4 on leukaemic cells of 12 children with an isolated medullary relapse (median WBC: 106×10^9/l; DFS: 13 months) and of 25 leukaemic children with a favourable outcome (median follow-up 60 months, range 24–165, median WBC $38{\cdot}3 \times 10^9$/l). Cells were stored in liquid nitrogen.

Phenotypes, according to the criteria of the European Group for the Immunological Characterization of Leukaemia (EGIL) (Bene *et al*, 1995), were distributed as follows: three pre-B1, 26 pre-B2, 10 pre-B3, one mature B and seven T-cell acute lymphoblastic leukaemia (T-ALL). CXCR-4 expression [Mean Fluorescence Intensity (MFI), percentage of positive cells] was detected by indirect immunofluorescence using an antibody against CXCR-4 (clone 12G5, R & D Systems, UK) as previously described (Sbaa-Ketata *et al*, 2001). Immunofluorescence analysis was performed on an EPICS-XL4 (Coulter, France) flow cytometer. In agreement with Crazzolara *et al* (2001), CXCR-4 was present on the majority of ALL blast cells, as only one case was considered to be negative according to the EGIL criteria. This patient had a favourable outcome. For the 46 cases with positive expression of CXCR-4, the median percentage of positive cells was 87·5% and the MFI was 3·81. No significant difference in CXCR-4 expression was observed between T and B-ALL subtypes.

We found a significant correlation between CXCR-4 expression and WBC count at diagnosis using the non-parametric Kendall test ($P = 0{\cdot}01$).

Patients were divided in two groups according to the presence or absence of splenomegaly and/or hepatomegaly at diagnosis. In agreement with Crazzolara *et al* (2001), patients with organ infiltration had a significantly higher expression of CXCR-4 than the group without tumoral symptoms (median MFI 4·29 and 2·47, respectively, $P < 0{\cdot}03$). We also compared the expression of CXCR-4 on leukaemic cells of patients without relapse, with those who experienced isolated medullary relapse or extramedullary relapse. Surprisingly, no significant difference could be detected between these three groups of patients (Fig 1). We then analysed the expression of CXCR-4 in two groups of children: those with a DFS under 24 months (12 cases) and those with a DFS over 24 months (34 cases). Children with a DFS under 24 months had a significantly higher expression of CXCR-4 (MFI 4·79) than children in first remission at 24 months (MFI 2·85, $P < 0{\cdot}02$). Ten patients presented a relapse after 24 months. They had similar levels of CXCR-4 (MFI 2·57) compared with patients in continuous first remission (MFI 2·91). Twelve children died: they had significantly higher levels of CXCR-4 (MFI 4·79) than surviving children (MFI 2·88, $P < 0{\cdot}02$).

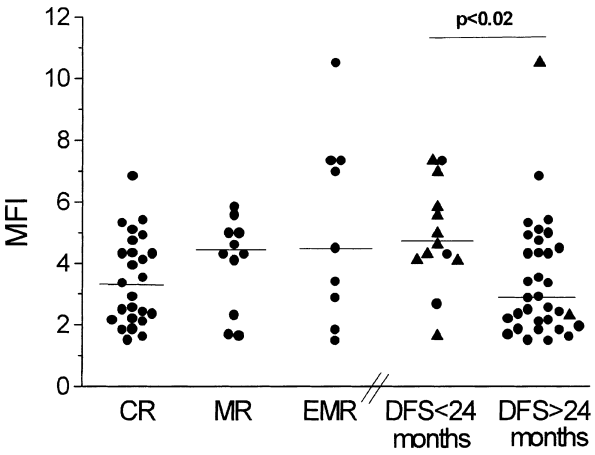

Fig 1. Distribution of CXCR-4 expression on leukaemic cells of children with newly diagnosed ALL. CR: complete remission, IMR: isolated medullary relapse, EMR: extramedullary relapse (associated or not with medullary relapse), DFS: disease free survival. Horizontal lines indicate the median value in each group (▲, deceased children).

These results do not confirm that higher levels of CXCR-4 constitute a prognostic factor of extramedullary relapse in children with ALL, but may be associated with a poorer outcome. The reasons of the discrepancy between the two studies are not clear: we used the same antibody to quantify CXCR-4 expression. We cannot exclude an influence of the treatment. In our study, all the children were treated by the French Acute Lymphoblastic Leukaemia Co-operative Group protocol (FRALLE 93), whereas several Berlin–Fankfurt–Munster (BFM) protocols were used in Crazzolara *et al* (2001). It is notable, however, that the sample size was small in both studies.

Despite these restrictions, the data obtained by Crazzolara *et al* (2001), as well as our data, suggest that CXCR-4 expression analysis in acute leukaemia cells could be of interest. The prognostic relevance of CXCR-4 requires further evaluation in large prospective studies of homogeneously treated patients.

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Keywords: CXCR-4, childhood ALL, relapse, prognosis.